


Dissociation of cells

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 An abbreviated version of this protocol was published in eLIFE in May 2021

Novel cell types and developmental lineages revealed by single-cell RNA-seq analysis of the mouse crista ampullaris

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Detailed protocol

1. Dissect cristae including the ampulla in ice cold Hank's buffered salt solution (HBSS; Cat. No. 14025–92; Thermo Fisher Scientific; Waltham, MA).
OPTION: to increase the proportion of sensory cells, dissect away the ampulla.
2. Pool dissected cristae and digested in 2% collagenase IV (Worthington, Cat. No. LS004186) at 37°C for 5 min.
3. Add a 10% vol of FBS and wash cristae in HBSS three times.
4. Digest cristae in 2% collagenase II (Worthington, Cat. No. LS004174) at 37°C for 30 min.
5. Add a 10% vol of FBS and wash cristae in HBSS three times.
6. Dissociate cristae to single cells in papain (Papain Dissociation Kit; Worthington, Cat. No. LK003150) for approximately 1 hr at 37°C with trituration every 10 min.
7. Stop dissociation by adding an equal volume of ovomucoid from the papain dissociation kit and wash cells in HBSS (spin at 400 g for 10 min. at 4°C recollect cells after each wash).
8. Strain cells through the 40 micron cell strainer and count with a hemocytometer.

This is not different than what is in the paper, currently we are working on getting a better dissociation protocol. I would like a faster dissociation so not so much time for gene expression changes. Embryonic tissue is much easier to dissociate but the tight junctions on the sensory part make that difficult at later stages. For the crista as opposed to other structures we found collagenase treatment a necessary step.

Olivia

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. McDonogh, O. B. and Wilkerson, B. A. (2021). Dissociation of cells. Bio-protocol Preprint. bio-protocol.org/prep1208.
2. Wilkerson, B. A., Zebroski, H. L., Finkbeiner, C. R., Chitsazan, A. D., Beach, K. E., Sen, N., Zhang, R. C. and Bermingham-McDonogh, O. (2021). Novel cell types and developmental lineages revealed by single-cell RNA-seq analysis of the mouse crista ampullaris. eLIFE. DOI: [10.7554/eLife.60108](https://doi.org/10.7554/eLife.60108)

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